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Abstract

Microbial communities living in *Sphagnum* are known to constitute early indicators of ecosystem disturbances, but little is known about their response (including their trophic relationships) to climate change. A microcosm experiment was designed to test the effects of a temperature gradient (15, 20 and 25°C) on microbial communities including different trophic groups (primary producers, decomposers and unicellular predators) in *Sphagnum* segments (0-3 cm and 3-6 cm of the *capitulum*). Relationships between microbial communities and abiotic factors (pH, conductivity, temperature, and polyphenols) were also studied.

The density and the biomass of testate amoebae in *Sphagnum* upper segments increased and their community structure changed in heated treatments. The biomass of testate amoebae was linked to the biomass of bacteria and to the total biomass of other groups added and, thus, suggests that indirect effects on the food-web structure occurred. Redundancy analysis revealed that microbial assemblages differed strongly in *Sphagnum* upper segments along a temperature gradient in relation to abiotic factors. The sensitivity of these assemblages made them interesting indicators of climate change. Phenolic compounds represented an important explicative factor in microbial assemblages and outlined the potential direct and/or indirect effects of phenolics on microbial communities.

Keywords: *Sphagnum*, testate amoebae, temperature gradient, phenolic compounds, bioindicators

Résumé

Les microorganismes des sphaignes sont connus comme indicateurs précoces des perturbations environnementales. Or, peu d'études portent sur l'influence de la température sur ces communautés. Dans cette étude, l'effet d'un gradient de températures (15, 20 et 25°C) sur les microorganismes des sphaignes, incluant différent groupes trophiques (producteurs primaires, décomposeurs, micro-prédateurs), a été testé dans les différentes parties des sphaignes (0 à 3 cm et 3 à 6 cm du *capitulum*). Les relations entre les microorganismes et les facteurs abiotiques (pH, conductivité, température, polyphénols) ont également été analysées.

La densité et la biomasse des amibes à thèque ont augmenté dans les parties supérieures des sphaignes. Une modification de la structure de leur communauté a aussi été mise en évidence. La biomasse des amibes à thèque est apparue liée à celle des bactéries et à la biomasse additionnée des autres groupes, suggérant des effets indirects de la température sur les relations trophiques. Les analyses par redondance ont révélé que la structure des communautés microbiennes variait entre les différents traitements dans les parties supérieures des sphaignes. Ainsi, les communautés microbiennes des sphaignes apparaissent comme un outil intéressant quant au suivi *in situ* des changements climatiques. Les polyphénols ont également été identifiés comme un facteur explicatif important de la structure des communautés microbiennes, montrant ainsi leurs effets potentiels sur les microorganismes des sphaignes.

Mots-clés : Sphaignes, amibes à thèque, gradient de température, composés phénoliques, bioindicateurs

Introduction

Climatic change models cover a wide range of temperature-increase scenarios (2°C up to 8°C), especially at high latitudes where the majority of *Sphagnum*-peatlands occurs (Gorham, 1991; IPCC, 2007). Peatlands are complex ecosystems, because of the diversity of habitats and micro-habitats allowing the establishment of diverse communities (Rydin and Jeglum, 2006). Studying the impact of climate change on these ecosystems remains particularly difficult and complex because of several biological and chemical parameters interacting (Davidson and Janssens, 2006). Some microcosm studies were elaborated to test the impact of a temperature increase on *Sphagnum* plant communities (Breuwer et al., 2008, 2009). However, such studies were carried out at the scale of simple organisms or populations and did not incorporate the interactions between different populations. Thus, a simplified ecosystem including different trophic levels could be then an interesting approach to better understand the effect of a temperature increase. In this context, the use of microbial communities living in *Sphagnum* represents a simplified ecosystem (Nguyen-Viet et al., 2007; Meyer et al., 2009). *Sphagnum* mosses are ubiquitous, cosmopolitan, and characterized peatlands (Shaw et al., 2003; Rydin and Jeglum, 2006). Moreover, *Sphagnum* mosses shelter a large number of microbial species belonging to the different trophic groups, including bacteria, algae, testate amoebae, ciliates, or fungi, which have a short generation time (hours up to few weeks) (Schönborn, 1986; Gilbert et al., 1998).

Some studies have demonstrated that the richness and density of single microbial groups (e.g. testate amoebae, microalgae) and/or the structure of microbial communities living in *Sphagnum* are affected by environmental perturbations such as nitrogen addition and atmospheric pollution (Howell and South, 1981; Gilbert et al., 1998; Mitchell et al., 2003; Nguyen et al., 2007, 2008). In addition, the integration of

104 testate amoebae into an experimental model is interesting because they feed on a wide
105 range of preys, (i.e. bacteria, fungi, organic matter, algae, other protozoa) and could
106 integrate ecosystem perturbations (Gilbert et al., 2000, 2003). Thus, such a simplified
107 system not only would notably give insight into the interactions between species but
108 also would incorporate biotic and abiotic factors (including phytochemicals)
109 interactions between *Sphagnum* and its associated microbial communities.

110 The potential phytochemical interactions between *Sphagnum* and microbial
111 communities are rarely studied. However, phenolic compounds (secondary metabolites)
112 produced by plants play an important role in the interaction of vegetation with its
113 environment (Hättenschwiler and Vitousek, 2000; Chiapusio et al., 2005). For example,
114 in humus spruce forests, such compounds are implicated in the increase of several
115 microbial communities (i.e. cellulose hydrolysers) and in the decrease of others (i.e.
116 bacteria) (Souto et al., 2000, 2001). Some studies have addressed phenolic production
117 by *Sphagnum* (e.g. Rasmussen et al., 1995), more specifically the phenolics weakly and
118 primarily bound to the cell wall (Verhoeven and Liefveld, 1997). Because of *Sphagnum*
119 morphology and anatomy, water-soluble phenolics can be easily released in *Sphagnum*
120 environments. Recently, Jassey et al. (2011) demonstrated that such compounds were
121 involved in the fine-scale microdistribution of testate amoebae along an ecological
122 gradient. A multitude of environmental factors cause significant shifts in the quantity of
123 phenolic compounds in vascular plants (e.g. UV-B; Spitaler et al., 2008), but they are
124 understudied in *Sphagnum*. The patterns of phenolic compounds at the surface of
125 *Sphagnum* layers may be susceptible to temperature increase and to interactions with
126 microbial communities living in *Sphagnum*.

127 In this study, we assessed the response of vertical patterns of microbial
128 communities living in *Sphagnum* and the relationships among microbial communities,

129 temperature, and total phenolic compounds along a temperature gradient. The
130 abundance and the structure of microbial communities and the concentrations of
131 phenolic compounds were quantified in *Sphagnum* peat cores placed at 3 different
132 temperatures (15, 20, and 25°C) after 8 weeks in a growth chamber. According to the
133 gradient of temperature, we addressed the following hypotheses: (*i*) that the structure
134 and biomass of microbial communities would vary among temperature treatments and
135 *Sphagnum* layers (upper and lower), (*ii*) that the effect of temperature would be
136 different for each microbial group according to their trophic position, and (*iii*) that total
137 phenolic compound concentrations quantified in *Sphagnum fallax* would change along
138 the temperature gradient and *Sphagnum* layers, and would explain a similar fraction of
139 the community data as other environmental factors.
140

141 **Materials and Methods**

142 *Sphagnum* sampling and experimental setup

143 *Sphagnum fallax* was collected in a peat bog in the Jura Mountains (Sur-les-Seignes,
144 Frambouhans-Les Ecorces, France, 47°18'N, 6°79'E) at an altitude of 846 m above sea
145 level on 19th October 2007 (temperature 10°C). The climate of the area is characterized
146 by cold winters (an average of −2.4°C in January) and mild summers (an average of
147 14.6°C in July). The annual mean temperature of the region is about 6.6°C. The annual
148 amount of precipitations is 1417 mm, and the duration of snow cover is an average of
149 50 day. year $^{-1}$. Surfaces of *S. fallax* as homogenous and pure as possible were selected.
150 Sixteen *Sphagnum* peat cores (25 cm x 15 cm) were randomly sampled with a knife.

151 Four peat cores were assigned in quadruplicates to described the initial stage (IS)
152 and 12 peat cores for the three treatments 15, 20 and 25°C (designated T15, T20 and

T25, respectively). A large gradient of temperature was chosen to observe the response of communities. Peat cores were randomly placed between the different levels of the growth chamber (Cryonext RTH600). General conditions selected were a relative humidity of > 70%, a light intensity of 120 $\mu mol.s^{-1}.m^{-2}$, and a photoperiod of 12h (light)/12h (dark). Each treatment corresponded to 1 level of the growth chamber. For each treatment, peat cores were placed in 1 big tray full of water (water level = 20 cm) and were humidified with a standard nutrient solution (Volvic water). The water depth was kept at 2 cm below the top of *Sphagnum* and readjusted with demineralized water every 2 days. The average relative humidity of *Sphagnum* was around 90% in *Sphagnum* segments for each treatment during the experiment. The 15°C temperature corresponded to the temperature of the growth chamber. The 20°C and 25°C temperatures were obtained by heating the water of the tray with respectively 2 thermo-divers of 50 and 200 W and 2 thermo-divers of 200 W. Moreover, aquarium pumps ($5L.min^{-1}$) were installed in the warm trays to obtain homogeneous temperatures. The position of peat cores was changed in each tray every 2 days to avoid experimental differences. The temperature was recorded several times a day during the experiment in each peat cores. The water chemistry (pH, conductivity and Eh potential) was also measured in each peat cores every 2 days.

Before and after 8 growing weeks 20 *S. fallax* were sampled in each peat cores and cut into 2 layers from the capitulum 0-3 cm (upper segments) and 3-6 cm (lower segments). For microbial analysis, the samples were fixed in 20 mL of glutaraldehyde (2% final concentration). No mosses were taken from the 5 cm bordering peat cores to avoid a border effect.

177 Total phenolic compounds assay

178 Phenolic compounds were extracted from lyophilized mosses following two methods as
179 described in Jassey et al. (2011) and commonly used in phytochemical interactions
180 (Waterman et al., 1994; Macheix et al., 2005). Water-soluble phenolics (hereafter "free
181 phenolics") were extracted using distilled water and corresponded to phenolics
182 susceptible to directly interact with microorganisms. Primarily bound phenolics
183 (hereafter "bound phenolics") were extracted using ethanol/distilled water solution
184 (80:20, v/v) and mainly corresponded to phenolic acids slightly bound to cell-wall.
185 Thereafter, free and bound total phenolic contents were quantified with the Folin-
186 Ciocalteau reagent and were expressed in milligrams of equivalent gallic acid (A_{760})
187 (Gallet and Lebreton, 1995).

188 Microbial Communities analyses

189 Microbial organisms were extracted from *S. fallax* with the standard method of Nguyen-
190 Viet et al. (2007). Each sample was shaken for 1 min on a vortex and then *Sphagnum*
191 mosses were pressed to extract microorganisms (first solution). The mosses were then
192 soaked again with 20 mL of glutaraldehyde (2% final concentration), shaken a second
193 time on a vortex, and pressed to extract *Sphagnum* leachate. The leachate was settled for
194 12h, and afterward the supernatant was added to the *Sphagnum* and the material on the
195 bottom was added to the first solution. The process was repeated 6 times, and all
196 fractions were combined to obtain a final composite sample of 40 mL. The remaining
197 fraction was dried at 80°C for 48h and weighted to express microbial density in grams
198 of dry weight of *Sphagnum*.

199 Primary producers, fungi and unicellular predators: a 3 mL subsample of the
200 final composite sample was settled for 2h in a plankton chamber and analyzed at x 400

magnification by inverted microscopy (Olympus IX71) following Uthermöhl's method (1958). The whole slide was analyzed and the different groups of cyanobacteria, microalgae, unicellular predators (flagellates, testate amoebae and ciliates) and fungi hyphae and spores were counted and measured. For testate amoebae, only living tests were counted.

Bacteria: a 1 mL subsample of the final composite sample was stained with 50 µL of 4,6 diamino-2-phenylindol (DAPI, 0.2% of final concentration) for 15 min in the dark, filtered through 0.2 µm black membrane filters, and examined by epifluorescence microscopy at x 1000 magnification (Porter and Feig, 1980). Bacteria numbers and size were estimated with an image analysis program (LUCIA 4.0). For each sample, between 8 and 35 photographic grips were observed. The number of bacteria cells counted and measured was situated between 514 and 1132. This direct counting using DAPI gave the total density of bacteria.

The biovolume of each community was estimated by assuming geometrical shapes and converted to carbon using the following conversion factors: bacteria, 1µm3 = 5.6×10^{-7}µgC (Bratbak, 1985); cyanobacteria and microalgae, 1 µm3= 1.2×10^{-7} µg C (Borsheim and Bratbak, 1987); ciliates and testate amoebae, 1 µm3 = 1.1×10^{-7} µg C (Weisse et al., 1990); fungi, 1 µm3 = 2.5×10^{-7} µg C (Gilbert et al., 1998). The data were expressed as micrograms of Carbon per gram of *Sphagnum* dry mass per square centimeter (µg C.(g DW)$^{-1}$.cm^{-2}).

Numerical analysis

Since the data distributions of microbial communities, phenolic compounds and water chemistry were not normal and statistically dependant, a non-parametric 2-way analysis of variance test (Friedman test) was used to determine the differences among the temperatures (T15, T20 and T25).

To reduce the influence of dominant taxa in the multivariate analysis, carbon biomass of microbial communities was transformed using the relation Ln(x+1) (Nguyen-Viet et al., 2007). Multiple factor analysis (MFA) was used to assess the general structure of the data and to determine the relationships among the 2 microbial community's data sets (upper and lower) and the 3 environmental variables data sets (water chemistry, temperature and phenolics). MFA was chosen because it allows the simultaneous coupling of several groups or subsets of variables defined on the same objects (Escofier and Pagès, 1994). The MFA was performed in 2 steps. Firstly, a first principal component analysis (PCA) was applied to the whole set of variables in which each subset was weighted by dividing all its elements by the first eigenvalue obtained from its PCA. Secondly, the normalized subsets were assembled to form a unique matrix and a second PCA was performed on this matrix. Euclidean distances of global PCA were then used in MFA to perform cluster analysis according to the Ward method, and the resulting dendrogram was projected in the MFA ordination space. This analysis revealed the main differences in the structure of the data described by all biotic and abiotic subsets of variables (Lamentowicz et al., 2010).

We assessed the relationships among the microbial communities in the upper and lower sampling depth and the 3 sets of environmental variables: (*i*) water chemistry (pH, Eh potential and conductivity), (*ii*) temperature and (*iii*) phenolic compounds (bound and free). The ordination patterns of microbial communities and their causal relationships to environmental data-sets were assessed using redundancy analysis (RDA) (Ter Braak and Simlauer, 1998). A forward selection of explanatory variables was computed for each of the *Sphagnum* segments, using automatic stepwise model building for constrained ordination methods ("ordistep" function). The proportion of variance explained by environmental variables was quantified using variance

partitioning. Adjusted R^2 were used in all RDA to estimate the proportion of explained variance by each environmental variable (Peres-Neto et al., 2006).

All multivariate analyses were performed with the software R (R Development Core Team, 2010) using the vegan (Oksanen et al., 2010) and FactoMineR (Husson et al., 2009) packages.

Results

Environmental variables

During the experiment, temperatures recorded were stable in both *Sphagnum* segments with 14.8 ± 0.4°C for T15, 19.4 ± 0.3°C for T20 and 23.9 ± 0.5°C for T25. The conductivity and the Eh potential were significantly lower ($P < 0.05$) in T15 (30 ± 9.4 $\mu S.cm^{-1}$ and 370.1 ± 16.2 mV, respectively) than in T20 and T25 (19.1 ± 3 $\mu S.cm^{-1}$ and 335.5 ± 20 mV, respectively). The pH was stable during the experiment (pH = 4.1 ± 0.5 in IS, T15, T20 and T25).

Temperature effect on total phenolic compounds

The concentrations in the 3 treatments were 1.5 times higher in upper segments (bound: an average of 2.6 $mg.g^{-1}$ DW, free: an average of 1.8 $mg.g^{-1}$ DW) than in lower segments (bound: an average of 1.6 $mg.g^{-1}$ DW, free: an average of 0.7 $mg.g^{-1}$ DW) (Table 1). Bound phenolics produced by *S. fallax* did not significantly differ between the upper and the lower segments, with the different temperatures. Free phenolics were significantly higher in upper segments ($P < 0.04$) for T25 than for the other treatments. A significant relation was also found between bound and free phenolics ($P < 0.001$, r = 0.62).

275 Microbial communities

276 The main change in the total biomass of microbial communities occurred in the upper
277 segments of *Sphagnum* whereas it was quite stable in lower segments (Fig. 1). Indeed
278 the total biomass in upper segments was nearly twice as high with elevated temperature
279 (T15: 1400 µg C.(g DW)$^{-1}$.cm^{-2}, T20 and T25: 1700 and 2700 µg C.(g DW)$^{-1}$.cm^{-2}, $P <$
280 0.01).

281 Primary Producers. The biomass of primary producers did not significantly vary
282 between T15, T20, and T25 (Fig. 1). The biomass of microalgae was 85 ± 31 µg C.(g
283 DW)$^{-1}$.cm^{-2} in upper segments and 106 ± 31 µg C.(g DW)$^{-1}$.cm^{-2} in lower segments. The
284 community was dominated by the same species along *Sphagnum* segments in any
285 treatments. *Chlorophyceae* (e.g. *Cylindrocystis* sp and *Penium* sp) and *Bacillariophycae*
286 (e.g. *Pinnularia viridis*) were the main identified genus. Cyanobacteria represented a
287 small proportion (< 5%) of the total biomass along *Sphagnum* segments in any
288 treatments with an average biomass of 50 ± 17 µg C.(g DW)$^{-1}$.cm^{-2} in upper segments
289 and 31± 10 µg C.(g DW)$^{-1}$.cm^{-2} in lower segments. Cyanobacteria were dominated by
290 *Chroococcales* (e.g. *Chroococcus giga*) and *Nostocales* (e.g. *Anabaenae cylindrica* and
291 *Aphanothece nidulans*) along *Sphagnum* segments.

292 Decomposers. The biomass of bacteria was 2 times higher in upper and lower
293 segments with elevated temperature (T20 and T25) than in T15. For example, bacterial
294 biomass increased from 200 µg C.(g DW)$^{-1}$.cm^{-2} in T15 to 440 µg C.(g DW)$^{-1}$.cm^{-2} at
295 T25 in upper segments ($P <$ 0.01; Fig. 1). Fungal biomasses were not significantly
296 different among treatments (70 µg C.(g DW)$^{-1}$.cm^{-2} in upper segments and 10 µg C.(g
297 DW)$^{-1}$.cm^{-2} in lower segments) (Fig.1).

298 Unicellular predators. The biomass of testate amoebae increased from T15 (380
299 µg C.(g DW)$^{-1}$.cm^{-2}) to T25 (600 µg C.(g DW)$^{-1}$.cm^{-2} $P <$ 0.01; Fig. 1). At the IS the

most frequent testate amoebae were *Archerella* (*Amphitrema) flavum* (29%), *Nebela tincta* (18%) and *Hyalosphenia papilio* (18%) in upper segments and *A. flavum* (30%), *N. tincta* (28%) and *Difflugia bacillariarum* (27%) in lower segments (Table 2). The same species were dominant at T15 with *H. papilio* (42%), *A. flavum* (16%), and *N. tincta* (16%) in upper segments, and *A. flavum* (29%), *E. strigosa* (27%), and *N. tincta* (16%) in lower segments. For T20-T25 communities, the upper segments were dominated by *H. papilio* (35% and 43%, respectively) and *E. strigosa* (30% and 27%, respectively) (Table 2). The biomass of testate amoebae was correlated with the total biomass of other groups added ($r = 0.63$, $P < 0.01$) in upper segments but not in lower segments ($r = 0.15$, $P = 0.57$). The density of *E. strigosa* was significantly correlated to the temperature increase ($r = 0.62$), the density of bacteria ($r = 0.49$) and free phenolics ($r = 0.58$) in upper segments of *Sphagnum* ($P < 0.01$). In lower segments, no species was significantly influenced by the temperature gradient nor free phenolics.

The results obtained for flagellates and ciliates strongly varied among treatments in upper and lower segments but no effect of temperature was clearly observed.

Relationships between environmental parameters and *Sphagnum* biota

The MFA of the 3 environmental matrices (water chemistry, temperature and phenolics) and the 2 microbial community (upper and lower segments) data sets confirmed the existence of an overall division among the temperature treatments (i.e. T15, T20 and T25) with different global structure of microbial communities (Fig. 2).

In the separate RDAs, T20 and T25 microbial assemblages and T15 microbial assemblage were clearly separated in upper segments (Fig. 3a), while in lower segments the separation between treatments was unclear (Fig. 3b). The models explained

respectively 56.4% and 27.6% of the variability in microbial data in upper and lower segments analysis ($P < 0.01$ for each axes, Monte-Carlo permutation test, 999 permutations). T20 and T25 microbial assemblages (particularly bacteria, fungi and testate amoebae) in upper segments were related with temperature and free phenolics, while microbial assemblage in T15 was related to Eh potential. In lower segments, microbial community structures along the temperature gradient were not clearly separated, except some T20 and T25 replicates correlated with elevated temperatures and the biomass of bacteria.

The successive RDAs on individual environmental variables revealed that the proportion of microbial data explained by each explanatory variable varied among variables both between the environmental data sets and along the 2 vertical positions (Table 3). The overall RDA (upper and lower segments) showed that free phenolics and temperature data sets explained, independently of the other 2 data sets, respectively 11.9% ($P = 0.004$) and 7.6% ($P = 0.02$) of the microbial data variance. However, the variance explained by these variables was however much higher in the partial RDA's of upper and lower segments (Table 3). For example, temperature explained 42.1% of the microbial data variance in upper segments ($P < 0.001$) and decreased to 2.5% in lower segments, while the variance explained by free phenolics increased from 5.2% to 15.7% ($P < 0.05$) between the upper and lower segments.

Discussion

Temperature gradient effect on microbial food web

This study is unique in that it addressed the effects of experimental high temperature gradient on microbial communities living in *Sphagnum*, including different trophic groups. MFA confirmed the existence of 3 microbial community assemblages along the temperature gradient (T15, T20, and T25 microbial assemblages) and the validity of using *Sphagnum* and its associated microbial communities as an indicator of climate change. Interestingly, the results revealed different behavior of the trophic groups related to the temperature gradient. The biomass of the microbial primary producers (microalgae and cyanobacteria) did not vary at any temperature while an increase of the biomass of the microorganisms (bacteria and testate amoebae) involved in the microbial loop was recorded (dissolved organic matter → bacteria → heterotrophic protists (ciliates, flagellates, testate amoebae) → macrozoorganisms; Gilbert et al., 1998).

Along the temperature gradient, the density and the biomass of testate amoebae in *Sphagnum* upper segments increased and their community structure changed in heated treatments. These results combined with the significant relation between the biomass of testate amoebae and the total biomass of other groups added suggested that indirect effects on the food-web structure occurred. The significant link between *Euglypha strigosa* and bacteria along the temperature gradient seemed to confirm this hypothesis. Indeed, *Euglypha* is described as bacterivorous in literature (Gilbert et al., 2000; Beyens et al., 2009). The increase of bacteria density in *Sphagnum* upper segments probably influenced the abundance of *E. strigosa*. Beyens et al. (2009) also observed similar results for the correlation between testate amoeba communities and temperature effect. For example, *Trachelocorythion* and *Euglypha* genera reacted

positively with heatwave and were linked to the increase of bacteria. The high density and high relative abundance of *H. papilio* in heated treatments also suggested indirect effects on the food-web structure, since this species feed on a wide range of prey, notably small eugliphids (Gilbert et al., 2000, Meisterfeld, 2000a, b). Conversely, the biomass and the density of *N. tincta* did not vary along the temperature gradient. This species was recognized to essentially feed on autotrophic microorganisms during summer (Gilbert et al., 2003), and the biomass of these groups was not affected by the temperature increase. Microbial primary producers (microalgae and cyanobacteria) living in *Sphagnum* are well known to be sensitive to water chemistry, and to N and P addition (Hooper, 1981; Howell and South, 1981; Gilbert et al., 1998).

Because testate amoebae are at the end of the microbial food web and they feed on a wide range of prey, their community integrates the variations of the food web due to environmental perturbations (Gilbert et al., 1998, 2003; Nguyen-Viet et al., 2007; Mitchell et al., 2008). Thus, they could be interesting candidates for monitoring the impact of elevated temperatures in *Sphagnum* peatlands. On the other hand, to deepen our knowledge about the changes in the microbial food web with elevated temperatures, complementary experiments focusing on the feeding habit progression of testate amoebae would be necessary.

Sphagnum biota – environmental factors relationships

Among environmental factors, temperature appeared as the principal factor that determines microbial community variations. Direct gradient analysis (RDA) revealed that in upper segments temperature, Eh potential and conductivity significantly explained a high proportion of microbial data set variations. This result was not surprising since water chemistry generally contributed to a change in microbial

community distribution that occurred at the top of *Sphagnum* carpet (Howell and South, 1981; Mitchell et al., 2003; Mieczan, 2009). Conversely, in lower segments, only free phenolics significantly explained microbial data variations. Studying both upper and lower segments along a temperature gradient demonstrated that the response of microbial communities to abiotic factors differed between *Sphagnum* segments. These results illustrate (*i*) how vertical gradients lead to ecological niches separations in *Sphagnum* and (*ii*) the potential influence of phenolic compounds on microbial community distribution.

The *Sphagnum* upper segments were characterized by a higher phenolic content (bound and free) compared to the lower segments, and constituted original results about phenolic repartition in *Sphagnum.* These results were in accordance with *Sphagnum* biology, given that *capitula* constituted the dominant life parts of *Sphagnum* where main metabolic processes occurred (Clymo and Hayward, 1982). The metabolism of phenolic compounds in *Sphagnum* was more important at the *capitulum* layer than in lower segments. The observed correlation between free and bound phenolic compounds demonstrated that their production by *Sphagnum* was linked and similar. However, only free phenolics were affected by elevated temperature through an increase in top segments. Such result suggested that phenolic content was also affected qualitatively by elevated temperature. Different kinds of phenolics (e.g. phenolic acids and flavonoids) are known to be present in the *Sphagnum* genus (Rudolph and Samland, 1985; Opelt et al., 2007). To better understand phenolic *Sphagnum* metabolism under elevated temperatures, it would be necessary to characterize both qualitatively and quantitatively the phenolic extracts from the different segments by using a high-pressure liquid chromatography technique.

Free phenolics were related to microbial communities and explained a large part of the variance observed, particularly in the lower segments. This relation outlines (*i*) the potential chemical interaction between *Sphagnum* and microbial communities living in *Sphagnum* and (*ii*) the role of such compounds in the structure of microbial communities regardless of their increase at 25°C in upper segments. Similar results were found with the testate amoebae community structure (Jassey et al., 2011). Nonetheless, the significant relation between *E. strigosa* and free phenolics in upper segments suggested a potential direct effect of these compounds on this species since its density did not increase in lower segments while the density of bacteria increased. Although this result does not allow a conclusion to be drawn on a possible direct positive effect of water-soluble phenolic compounds on *E. strigosa*, it raises the issue of the possible role of such compounds. Recently, *p*-hydroxyl phenolic acids released by *Sphagnum* mosses have been shown to possess antibacterial and antifungal activity (Opelt et al., 2007; Mellegård et al., 2009). Thus it is possible that water-soluble phenolic compounds released by *Sphagnum* play a role in microbial assemblages through direct (e.g. physiological) and/or indirect (e.g. through impact on prey−predator relationships) effects.

Conclusion

This original approach of studying *Sphagnum* upper and lower segments revealed a strong relationship between microbial community structures and rising temperatures in upper segments of *Sphagnum*. Because of the different biotic and abiotic gradients in *Sphagnum*-dominated peatlands, our study highlighted the need to study together different microbial groups along the living parts of *Sphagnum*. A destabilization of the

444 microbial food web by elevated temperatures (5°C) through their trophic relationships is
445 suggested in upper segments. Therefore, microbial assemblages and/or testate amoebae
446 may be useful indicator to monitor climate change in peatlands. The relationships
447 between water-soluble phenolics compounds and microbial communities living in
448 *Sphagnum* remained an interesting result. Further *in situ* investigations characterized by
449 a realistic warming and moisture variations should be undertaken.

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451

452 **Acknowledgments**

453 This article is a contribution of the Agence National de la Recherche (ANR)
454 PEATWARM project (Effect of moderate warming on the functioning of *Sphagnum*
455 peatlands and their function as carbon sink). PEATWARM is supported by the French
456 National Agency for Research under the "Vulnerability: Environment—Climate"
457 Program (ANR-07-VUL-010). V. E. J. Jassey has been supported by a grant from the
458 Franche-Comté Region.

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Tables

Table 1. Total concentrations ($mg.g^{-1}$ DW) of *Sphagnum fallax* free and bound phenolics in two layers (upper and lower segments) at initial stage (IS) and at the temperatures 15, 20, and 25°C (T15, T20, and T25) (n = 4). *Letters* indicate significant differences of the total phenolic concentrations between the upper and the lower segments for a same treatment ($P < 0.05$) and *Asterisk* indicates significant difference between temperatures ($P < 0.05$).

Table 2. Abundance of 12 testate amoebae species found in upper and lower segments of *Sphagnum fallax* at initial stage (IS) and grown over an 8 weeks period at 15, 20 and 25°C (T15, T20, and T25) (n=4). *Asterisks* indicate significant differences of a same species related to the gradient of temperature in upper and lower segments ($P < 0.05$).

Table 3. Summary of redundancy analyses (RDA) on microorganisms and environmental variables: fraction of variance explained (%) and significance of individual variables taken alone or grouped.

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644 **Figures**

645

646 **Figure 1**. Total carbon biomass (µgC.g^{-1}.cm^{-2}, mean ± S.E.) of all microbial groups and
647 Carbon biomass (µgC.g^{-1}.cm^{-2}, mean ± S.E.) of microbial groups in *Sphagnum* samples
648 analyzed after 8 weeks related to the gradient of temperature. *Letters* indicate significant
649 differences of biomass between temperatures in upper and lower segments (Friedman
650 test, $P < 0.01$). IS, initial stage

651 **Figure 2**. Multiple factor analysis (MFA) of the two *Sphagnum* biota communities and
652 environmental (chemical, physical and phenolics) data sets. Biplot of axes 1 and 2 (both
653 significant at $P = 0.001$) is given together with the result of a hierarchical agglomerative
654 clustering (grey solid lines) obtained by the Ward method on the Euclidean distance
655 matrix between MFA site scores, showing three main groups of temperature treatment
656 (white symbols = T15, black symbols = T20, grey symbols = T25).

657 **Figure 3.** Redundancy analyses (axes 1 and 2) of microbial data in upper segments (a)
658 and lower segments (b). Environmental variables retained after model selection are
659 represented by arrows. Microbial groups are represented by dotted lines. F_phe : free
660 phenolics; Temp: temperature.

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